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Bioorganic & Medicinal Chemistry Letters 15 (2005) 2221–2224
Bioorganic & Medicinal Chemistry Letters

3-Acyl-2,6-diaminopyridines as cyclin-dependent kinase inhibitors: synthesis and biological evaluation

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Received 15 February 2005; revised 4 March 2005; accepted 7 March 2005

Abstract—A novel series of 2,6-diamino-3-acylpyridines were designed and synthesized as cyclin-dependent kinase (CDK) inhibitors. The representative compounds **2r** and **11** showed potent CDK1 and CDK2 inhibitory activities and inhibited cellular proliferation in HeLa, HCT116, and A375 tumor cells.

Cyclin-dependent kinases (CDKs), such as CDK1, CDK2, and CDK4, constitute a class of serine–threonine protein kinases that plays an important role in regulation of the cell cycle.[1] Abnormal CDK control of the cell cycle has been strongly linked to the molecular pathology of cancer. CDKs have thus become attractive therapeutic targets for cancer therapy.[2] The CDKs regulate cell cycle progression through complexes with their corresponding cyclin partners such as cyclin A, B, D, and E. For example, CDK1 associated with cyclin B regulates the cell cycle at the G2 and mitosis (cell division) phases. CDK1 inhibitors could block mitosis entry and arrest cell growth, and therefore may be useful therapeutic agents with potentially fewer side effects than conventional cytotoxic drugs targeting DNA synthesis.

Several CDK inhibitors have entered clinical evaluation for the treatment of cancer.[3] These include flavopiridol, 7-hydroxystaurosporine (UCN-01), roscovitine (CYC202), and an aminothiazole compound (BMS-387032).[4] In our program to develop CDK inhibitors as anti-cancer agents, we recently discovered that acyl-substituted diaminotriazole derivatives such as RWJ-387252 (**1**) are potent and selective CDK inhibitors and anti-proliferative agents.[5] To investigate the role of the central heterocyclic core in the biological activity of the acyl-diaminotriazoles, we designed a new series of 3-acyl-2,6-diaminopyridines (**2**) as potential CDK inhibitors. Herein we report the synthesis of these novel compounds and their biological evaluation as inhibitors of CDK activity and tumor cell proliferation.

Synthesis of 3-acyl-N^6-aryl-2,6-diaminopyridines (**2**) unsubstituted at the 4-position is outlined in Scheme 1. The key steps involved *ortho*-metalation–acylation of an *N*,*N*-diprotected 2,6-diaminopyridine, followed by nucleophilic substitution on activated aryl or heteroaryl halides with the less sterically-hindered 6-amino group of the deprotected 3-acyl-2,6-diaminopyridine products (**3**). Specifically, *ortho*-metalation–acylation of 2,6-bis-(pivaloylamino)pyridine (**4**)[6] was achieved by first treating with *n*-butyl lithium at −78 °C in *tert*-butyl methyl ether (MTBE) containing (*N*,*N*,*N*′,*N*′-tetramethyl)ethylenediamine (TMEDA), followed by coupling with an acyl chloride. Subsequent hydrolysis of the pivaloyl protecting groups with aqueous KOH afforded the 3-acyl-2,6-diaminopyridines (**3**). Selective nucleophilic substitution of aryl iodides or bromides with the 6-amino group of **3** was generally performed under palladium-catalyzed conditions to give the desired compounds (**2a**–**b**, **2e**, **2j**–**l**, and **2y**).

Keywords: Synthesis; Cyclin-dependent kinase inhibitor; CDK inhibitor; Cell proliferation inhibitor.
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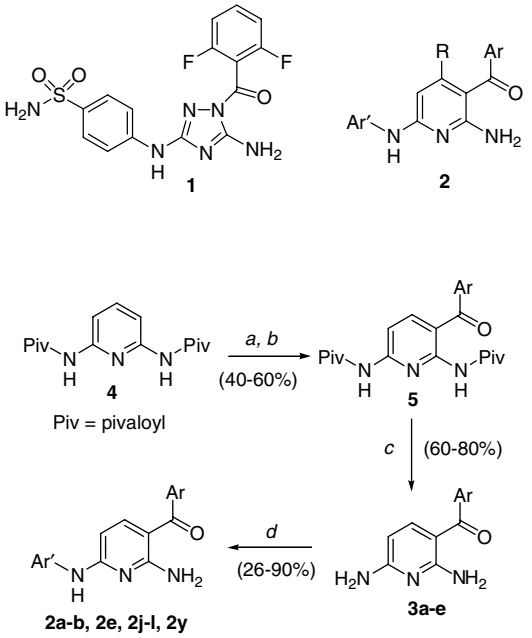
doi:10.1016/j.bmcl.2005.03.024

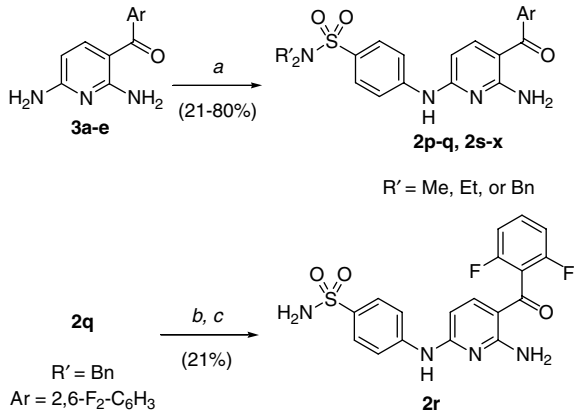


Scheme 1. General synthetic route toward 2,6-diamino-3-acylpyridines. Reagents and conditions: (a) *n*-BuLi, MTBE/TMEDA, −15 to 0 °C, 16 h; (b) ArC(O)Cl, 0 °C, 3 h; (c) 1:1 2 M KOH/dioxane, reflux, 6 h; (d) Ar′-I or Ar′-Br, $Pd_2(dba)_3$, BINAP, Cs_2CO_3, dioxane, 95 °C.

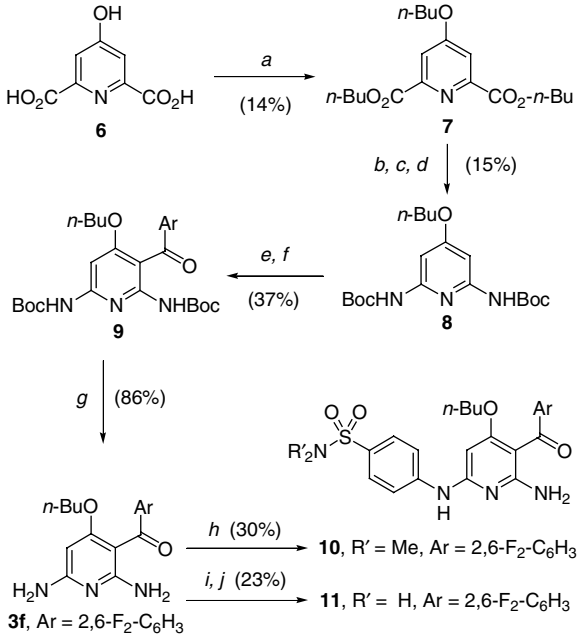
Cyano and nitro groups on **2b**, **2e**, and **2l** were further derivatized. For example, hydrolysis of nitrile **2b** with hydrochloric acid afforded amide **2c** (23%) and acid **2d** (27%). Catalytic hydrogenation of nitro compounds **2e** and **2l** gave the corresponding amine compounds **2f** (90%) and **2m** (46%). The amino group of **2f** was selectively derivatized by acetyl chloride, benzoyl chloride, and benzenesulfonyl chloride to give **2g** (82%), **2h** (99%), and **2i** (40%), respectively. Similarly, reductive amination of **2m** with ammonium formate and reaction with benzenesulfonyl chloride afforded compounds **2n** (14%) and **2o** (73%), respectively.

Because many potent CDK inhibitors contain a benzenesulfonamide group,[5a,7,8] this moiety was incorporated to the 3-acyl-2,6-diaminopyridine scaffold. Aromatic nucleophilic substitution of *para*-iodo- or *para*-bromobenzenesulfonamide with (2,6-diamino-pyridin-3-yl)-(2,6-difluoro-phenyl)-methanone (**3a**) using the palladium-catalyzed conditions described above failed to give the amination product. Nevertheless, *N*,*N*-dialkyl *para*-iodobenzenesulfonamides, including *N*,*N*-dimethyl, *N*,*N*-diethyl, and *N*,*N*-dibenzyl derivatives,[9] were successfully coupled with 3-acyl-2,6-diaminopyridines (**3a**–**e**) via the palladium-catalyzed amination protocol (Scheme 2). Stepwise deprotection of the benzyl groups of **2q** to give free sulfonamide **2r** was achieved in 21% combined yield by treatment with BBr_3 in methylene chloride, followed by aqueous HI in acetic acid.

To evaluate the effect of introducing an additional functional group on the scaffold, substitution at the 4-position of the core pyridine ring was investigated. The synthesis of 4-substituted diamino pyridine analogues is outlined in Scheme 3. Chelidamic acid (**6**) was first



Scheme 2. General synthetic route toward 2,6-diamino-3-acylpyridine with benzenesulfonamide group. Reagents and conditions: (a) 4-I-$C_6H_4SO_2NR'_2$,[9] $Pd_2(dba)_3$, BINAP, Cs_2CO_3, dioxane, 95 °C; (b) BBr_3, CH_2Cl_2, reflux, 6 h; (c) aqueous HI, HOAc, reflux, 4 h.



Scheme 3. General synthetic route toward 4-substituted 2,6-diamino-3-acyl-pyridine analogs. Reagents and conditions: (a) excess *n*-BuI, K_2CO_3, DMF; (b) N_2H_4, EtOH; (c) HCl, $NaNO_2$, 0 °C, 1 h; (d) *t*-BuOH, reflux, 5 h; (e) *n*-BuLi, MTBE/TMEDA, −15 to 0 °C, 16 h; (f) ArCOCl, 0 °C, 3 h; (g) 1:1 DCM/TFA, rt, 5 h; (h) 4-I-$C_6H_4SO_2NMe_2$,[9] $Pd_2(dba)_3$, BINAP, Cs_2CO_3, dioxane, 95 °C; (i) 4-I-$C_6H_4SO_2NHBoc$,[12] $Pd_2(dba)_3$, BINAP, Cs_2CO_3, dioxane, 95 °C; (j) 1:1 DCM/TFA, rt, 3 h.

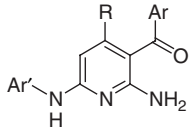
converted to the corresponding di-*n*-butyl ester *n*-butyl ether (**7**) in the presence of excess iodobutane (14%). Diphenyl phosphorylazide (DPPA)-mediated double Curtius rearrangement[10] using the diacid hydrolysis product of intermediate **7** proved to be problematic. An alternative stepwise procedure was then employed.[11] Toward that end, the diester ether **7** was first converted to the corresponding dihydrazide using hydrazine. The dihydrazide was then carefully converted to diazide at low temperature using nitrous acid generated in situ. The resulting diazide was then transformed to the

BOC-protected diaminopyridine **8** by heating at reflux in anhydrous *tert*-butanol (15%). *ortho*-Metalation–acylation at the 3-position of bis-Boc-protected 2,6-diaminopyridine **8** followed by deprotection afforded 3,4-disubstituted diaminopyridine **3f** (32% for two steps). Aromatic nucleophilic substitution of *N*,*N*-dimethyl-*para*-iodobenzenesulfonamide with compound **3f** gave compound **10** (30%). Similarly, palladium-catalyzed amination of *N*-Boc-*para*-iodobenzenesulfonamide[12] with compound **3f** followed by facile deprotection of the Boc group provided **11** (23%).

Table 1 shows the structures and CDK1 inhibitory activities for the 3-acyl-2,6-diaminopyridine analogues. Examination of substitution on the 6-anilino group reveals that the *ortho*-position (compounds **2e**–**i**) is the most sensitive site for maintaining CDK1 potency compared to the *meta* and *para* positions. The *para*-position is generally tolerant of the size and electronic characteristics of the substituent, except in the case of very large groups like *N*,*N*-dibenzylaminosulfonyl (**2q**) and *N*,*N*-diethylaminosulfonyl (**2u** and **2w**). With 2,6-difuorobenzoyl at 3-position of the core pyridine ring, aminosulfonyl (**2r**) and *N*,*N*-dimethylaminosulfonyl groups (**2p**) in the *para*-position of the N^6-phenyl ring produce the most potent CDK1 inhibitors. Also, replacement of the unsubstituted phenyl ring of **2a** with a 2-pyridinyl group (**2y**) improves CDK1 potency two-fold. On the other hand, replacement of the 2,6-difluorobenzoyl substituent of compound **2p** with 2-furoyl or 2-thienoyl groups (i.e., **2v** or **2x**) or removal of one or both fluorines (i.e., **2s** or **2t**) is detrimental to CDK1 potency.

As shown by the CDK1 data for compounds **3f**, **10**, and **11**, the 4-position at the pyridine core tolerates a lipophilic *n*-butoxy substituent, indicating that this could be another location to introduce additional functional groups to improve kinase inhibitory potency or ADME properties. Interestingly, the intermediate N^6-unsubstituted 3-benzoyl-2,6-diaminopyridines (**3a**–**c**, **3f**)

Table 1. Structures and CDK1 inhibitory activity for the 2,6-diamino-3-acyl-pyridine analogs



2a-y, **3a-f**, **10**,**11**

Compd #	Ar	Ar′	R	CDK1 IC_{50}, μM[a]
2a	2,6-F_2–C_6H_3	Ph	H	21.7
2b	2,6-F_2–C_6H_3	4-NC–C_6H_4	H	1.37
2c	2,6-F_2–C_6H_3	4-H_2NCO–C_6H_4	H	0.95
2d	2,6-F_2–C_6H_3	4-HO_2C–C_6H_4	H	~10
2e	2,6-F_2–C_6H_3	2-O_2N–C_6H_4	H	>10
2f	2,6-F_2–C_6H_3	2-H_2N–C_6H_4	H	11.6
2g	2,6-F_2–C_6H_3	2-AcHN–C_6H_4	H	>100
2h	2,6-F_2–C_6H_3	2-PhC(O)HN–C_6H_4	H	>100
2i	2,6-F_2–C_6H_3	2-PhS(O_2)HN–C_6H_4	H	>100
2j	2,6-F_2–C_6H_3	3-O_2N–C_6H_4	H	2.67
2k	2,6-F_2–C_6H_3	3-Cl–C_6H_4	H	6.16
2l	2,6-F_2–C_6H_3	4-O_2N–C_6H_4	H	0.96
2m	2,6-F_2–C_6H_3	4-H_2N–C_6H_4	H	0.75
2n	2,6-F_2–C_6H_3	4-MeHN–C_6H_4	H	1.14
2o	2,6-F_2–C_6H_3	4-PhS(O_2)HN–C_6H_4	H	0.74
2p	2,6-F_2–C_6H_3	4-$Me_2NS(O_2)$–C_6H_4	H	0.59
2q	2,6-F_2–C_6H_3	4-$Bn_2NS(O_2)$–C_6H_4	H	>10
2r	2,6-F_2–C_6H_3	4-$H_2NS(O_2)$–C_6H_4	H	0.36
2s	2-F–C_6H_4	4-$Me_2NS(O_2)$–C_6H_4	H	1.96
2t	Ph	4-$Me_2NS(O_2)$–C_6H_4	H	2.19
2u	Ph	4-$Et_2NS(O_2)$–C_6H_4	H	>100
2v	2-Furyl	4-$Me_2NS(O_2)$–C_6H_4	H	10.6
2w	2-Furyl	4-$Et_2NS(O_2)$–C_6H_4	H	>100
2x	2-Thienyl	4-$Me_2NS(O_2)$–C_6H_4	H	12.7
2y	2,6-F_2–C_6H_3	2-Pyridinyl	H	12.3
3a	2,6-F_2–C_6H_3	H	H	2.6
3b	2-F–C_6H_4	H	H	8.2
3c	Ph	H	H	1.1
3d	2-Furyl	H	H	>100
3e	2-Thienyl	H	H	>100
3f	2,6-F_2–C_6H_3	H	*n*-BuO	1.2
10	2,6-F_2–C_6H_3	4-$Me_2NS(O_2)$–C_6H_4	*n*-BuO	1.0
11	2,6-F_2–C_6H_3	4-$H_2NS(O_2)$–C_6H_4	*n*-BuO	0.26

[a] See Ref. 5a for a description of the CDK1 assay. IC_{50} data are the average of at least two separate experiments. IC_{50} values listed as >10 or >100 indicate no observed 50% inhibition at the highest dose tested, nor was an inhibition maximum observed.

Table 2. Inhibitory activity on other kinases and anti-proliferative activity on various tumor cells for representative compounds **2p**, **2r**, and **11**

Kinase or tumor cell	Inhibition IC_{50}[a] (μM)		
	2p	**2r**	**11**
CDK1	0.59	0.36	0.26
CDK2	0.50	0.18	0.084
VEGF	>100	>100	>100
HER2	>100	>100	>100
EGFR	>100	>100	>100
HeLa	>10	17.2	10.7
HCT116	>10	16.5	6.7
A375	>10	6.5	2.9

[a] See Ref. 5a for descriptions of kinase and cellular anti-proliferation assays. IC_{50} data are the average of at least two separate experiments. IC_{50} values listed as >10 or >100 indicate no observed 50% inhibition at the highest dose tested, nor was an inhibition maximum observed.

maintain modest CDK1 potency whereas CDK1 IC_{50} values for similar 2-furoyl (**3d**) and 2-thienoyl (**3e**) analogues could not be determined at the highest dose tested. In addition, the enhancement of CDK1 potency by 2,6-difluoro substituents was observed for the subset of compounds **2** having the aryl group at N-6; a similar trend was not seen in the subset of N^6-unsubstituted intermediate compounds **3**.

Table 2 shows a comparison of CDK1 with CDK2 and other kinase inhibitory activities as well as in vitro anti-proliferative activities in human tumor cells for three representative compounds (**2p**, **2r**, and **11**). These compounds are more potent against CDK2 than CDK1 and are inactive against VEGF-R2, HER2, and EGFR kinases at the highest concentration tested. Compounds **2r** and **11** also proved to be active in vitro as anti-proliferatives in various human tumor cell lines, such as HeLa (cervical carcinoma), HCT116 (colon carcinoma), and A375 (melanoma).

In summary, we have discovered a novel series of 3-acyl-2,6-diaminopyridine derivatives that are effective cyclin-dependent kinase inhibitors. The key steps for the synthesis employed an *ortho*-metalation–acylation of diprotected 2,6-diaminopyridines and a selective palladium-catalyzed amination on activated aryl or heteroaryl halides using the intermediate 3-acyl-2,6-diaminopyridines. Representative compounds **2r** and **11** showed potent CDK1 and CDK2 inhibitory activities and inhibited in vitro cellular proliferation in HeLa, HCT116, and A375 human tumor cell lines. Future progress on related series will be reported in due course.

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